Pestic. Sci. 1998, **53**, 209–216

Glutathione Transferases and Herbicide Detoxification in Suspension-Cultured Cells of Giant Foxtail (*Setaria faberi*)

Pamela J. Hatton,[1]‡ Ian Cummins,[1] Lindsey J. Price,[2]§ David J. Cole[2] & Robert Edwards[1]*

[1] Crop Protection Group, Department of Biological Sciences, University of Durham, Durham DH1 3LE, UK
[2] Rhône-Poulenc Agriculture Ltd, Ongar, Essex CM5 OHW, UK

20 October 1997; revised version received 12 January 1998; accepted 12 February 1998)

Abstract: Glutathione transferases (GSTs) catalysing the conjugation of 1-chloro-2,4-dinitrobenzene, the chloro-*s*-triazine herbicide atrazine, the chloroacetanilide herbicides metolachlor and alachlor and the diphenyl ether herbicide fluorodifen have been identified in suspension-cultured cells derived from the grass weed giant foxtail (*Setaria faberi* Herrm.). In contrast to suspension-cultured cells of maize, where atrazine-conjugating GSTs are lost during de-differentiation, the GSTs active toward this herbicide in *S. faberi* plants were also expressed in cultures, suggesting that these isoenzymes are subject to different regulation in the crop and weed. As a result, glutathione conjugation was the major route of atrazine metabolism in *S. faberi* cultures. Activities of these GSTs were maximal three days after sub-culturing when the cells were dividing most actively, when they were determined to be in the order CDNB > alachlor > metolachlor = fluorodifen > atrazine. This indicated that GSTs which are enhanced during cell division can metabolise herbicides. On the basis of activity per mg protein, GST activities in the cultures were between 20 and 60-fold higher than those determined in the foliage of *S. faberi* seedlings. The GSTs with activity towards CDNB were resolved into three peaks following anion-exchange chromatography at pH 7·8 using Q-Sepharose. Peak 1 GSTs were not retained, while peak 2 and peak 3 were sequentially resolved with an increasing concentration of salt. Peak 1 GSTs showed activity toward metolachlor and atrazine but showed little activity toward fluorodifen. Peak 2 and peak 3 GSTs were active toward atrazine and metolachlor, with peak 3 being particularly associated with activity toward fluorodifen. The GSTs in these peaks were then further purified using *S*-hexyl-glutathione-agarose affinity chromatography. In each case, the affinity-bound fraction of the GSTs consisted of 28 kDa and 26 kDa polypeptides, suggesting that the GST isoenzymes in *S. faberi* cultures are composed of related subunits. Our results demonstrate that the GST isoenzymes involved in herbicide metabolism in suspension cultures of a grass weed show a similar level of complexity to that determined in maize cell cultures. © 1998 SCI

Pestic. Sci., **53**, 209–216 (1998)

Key words: herbicide detoxification; atrazine; glutathione conjugation; glutathione transferases; plant cell cultures; maize; weeds; *Setaria faberi*

* To whom correspondence should be addressed.
Present addresses: ‡ Pesticides Safety Directorate, MAFF, York, YO1 2PX, UK;
§ School of Molecular and Medical Biosciences, University of Wales, College of Cardiff, Carduff, CF1 1ST, UK.
Contract/grant sponsor: Environmental and Physical Sciences Research Council.
Contract/grant sponsor: Biotechnology and Biological Sciences Research Council.
Contract/grant sponsor: Rhône-Poulenc Agriculture Ltd.

 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

1 INTRODUCTION

Suspension-cultured plant cells may be used as model systems for the rapid screening of herbicidal compounds and can be a useful tool for determining the phytotoxicity and metabolism of compounds *in vivo*.[1,2] The lack of organised tissues and the favourable ratio of the volume of cells to medium means that uptake and translocation are not limiting factors and toxicity and metabolism can be assayed directly with relatively small amounts of pesticide. The use of plant-tissue culture to study the metabolism of pesticides has been reviewed previously, though the enzymes responsible have only been characterised in a limited number of cases.[1,2]

Glutathione transferases (GSTs, EC 2.5.1.18) able to catalyse the conjugation of electrophilic herbicides with the tripeptide glutathione have been reported in many plant species.[3] GSTs can be major determinants in selectivity, with the tolerant crop containing higher levels of isoenzymes involved in herbicide detoxification than susceptible weeds.[3] However, although progress is now being made in characterising the GSTs in whole plants of crop and weed species,[3] far less is known regarding these enzymes in the corresponding plant cells grown in de-differentiated cultures. Such studies can be helpful in characterising GSTs which are not very abundant in whole plants and in determining their regulation. Suspension cultures of maize,[4–6] soybean,[7,8] pumpkin,[9] and tobacco,[10] all contain GSTs, although only the enzymes of soybean[7] and maize (Black Mexican Sweetcorn)[4–6] have been shown to have activity toward herbicides. Extracts from soybean cultures showed GST activities toward a range of herbicides including the diphenyl ethers fomesafen and acifluorfen, the chloroacetanilide metolachlor and the sulphonyl urea chlorimuron-ethyl.[7] With all these herbicides, the GST activities in the cultured cells were considerably higher than those determined in soybean plants. In contrast, in maize cultures, although GST activity toward metolachlor was higher than that determined in plants, activity toward atrazine was absent.[4,5] These cell cultures were unable to metabolise atrazine by glutathione conjugation, while in whole plants such conjugation was the major route of detoxification of this herbicide.[4] The GSTs in maize cultures with activity toward metolachlor have been characterised in some detail.[4–6] When resolved by anion-exchange chromatography, four peaks of activity toward metolachlor could be determined in cultures.[6] Following treatment with the herbicide safener benoxacor, the GST activities associated with these peaks were increased and another two peaks of activity were determined. Based on immunological studies, two of these isoenzymes were shown to contain polypeptides which were recognised by an antiserum raised to the *Zm* GST I-III heterodimer.[6] Thus, overall, the range of GSTs with activity toward chloroacetanilide herbicides appears to be very similar to that for the GSTs described in maize plants.[11,12] In recent studies we have been interested in comparing the GSTs involved in herbicide metabolism in maize and associated competing weeds,[13] with particular reference to the GSTs of giant foxtail (*Setaria faberi* Herrm.).[14] We have determined that *S. faberi* plants contain GSTs capable of metabolising chloroacetanilide and chloro-*s*-triazine herbicides.[13,14] In seedlings, these GST activities are much lower in *S. faberi* than in maize, but as the plants grow older these differences are lost, coinciding with the loss in herbicide selectivity.[14]

As an extension of our studies with whole plants, it became of interest to establish suspension cultures of *S. faberi*, examining their complement of herbicide-metabolising GSTs and their value as a source for the characterisation and purification of these enzymes. In view of the previous results obtained with Black Mexican Sweetcorn cultures,[4,5] it was also of interest to determine whether or not the *S. faberi* cell cultures could use GSTs in atrazine metabolism *in vivo*.

2 MATERIALS AND METHODS

2.1 Plants and suspension cultures of *Setaria faberi*

Seeds of *S. faberi* were obtained from Herbiseed Ltd (Wokingham, UK). Plants were grown in an environmental growth room under the conditions described previously.[14] For cell-culture studies, mature seeds were immersed in a sterilising solution (50 ml) containing sodium hypochlorite (1 g litre^{-1}) and a drop of 'Teepol' surfactant. After agitating on an orbital shaker (10 min), the seeds were repetitively washed with sterile distilled water and then placed onto Murashige and Skoog basal medium,[15] supplemented with sucrose (20 g litre^{-1}) 2,4-dichlorophenoxy acetic acid (2 mg litre^{-1}) and agar (8 g litre^{-1}) and incubated in the dark at 27°C. After two to three weeks, small, dark, mucilaginous calli were formed and these were removed and transferred onto fresh medium. Calli were then sub-cultured a further three times at three-week intervals until a secondary dense, white and rapidly dividing callus was observed. This white, friable callus was transferred into the supplemented Murashige and Skoog liquid medium and incubated on an orbital shaker (130 rev min^{-1}) in the light at 27°C. The suspension cultures (50 ml) were then sub-cultured every seven days using an 8 ml inoculum. When required, suspension-cultured cells were filtered under vacuum through Whatman No. 1 filter paper and then frozen under liquid nitrogen and stored at − 80°C.

2.2 Metabolism studies with [^{14}C]atrazine

Suspension-cultured cells (50 ml) were grown for three days after sub-culturing and fed with 50 µl of methanol containing [U-*triazinyl*-^{14}C]atrazine (288·6 MBq mmol^{-1}, 2·3 mM), which had been purified by TLC prior to use (97% purity).[13] Control treatments consisted of cells treated with methanol alone. At daily intervals, the cultures were harvested in duplicate and the medium and cells separated by vacuum filtration. The cell medium was adjusted to pH 2 by the addition of trifluoroacetic acid (2 g litre^{-1}) and after noting the volume, duplicate 200-µl samples were mixed with 4 ml scintillation fluid (Ecoscint, National Diagnostics) and the radioactivity present quantified by liquid scintillation counting. The medium was then applied to 20-ml lots onto an octadecyl sample preparation column (Alltech). The majority of the radioactivity (93 ($\pm$5)%, $n = 8$) was retained on the column and recovered with a wash with methanol (5 ml). After concentration under a stream of nitrogen, 20 µl of the methanolic extract was analysed by thin-layer chromatography (TLC).

The cells were weighed and homogenised in 10 volumes of methanol using a pestle and mortar and the extract filtered through Whatman No. 1 filter paper. After noting the total volume, duplicate 100 µl samples were assayed by liquid scintillation counting and the remaining extract concentrated to dryness under vacuum, prior to redissolving in methanol (200 µl) and applying 20 µl to a TLC plate. The cell debris remaining following solvent extraction was digested with tissue solubiliser (Protosol, NEN) as recommended by the manufacturer, prior to scintillation counting.

For TLC, aluminium-backed analytical plates, precoated with 0·2 mm silica gel 60 F_{254} (Merck, Darmstadt, Germany), were used and plates developed using butan-1-ol + acetic acid + water (4 + 1 + 1 by volume) or chloroform + ethanol (9 + 1 by volume). Radioactive metabolites were then located by autoradiography and identified by comparison with authentic standards as described previously prior to quantification by scintillation counting.[13]

2.3 Purification and characterisation of GSTs

During their purification, GST activities toward 1-chloro-2,4-dinitrobenzene (CDNB) and the herbicides were determined as described previously.[13] Protein concentrations were determined using the Bio-Rad dye-binding assay reagent with γ-globulin as the reference protein as recommended by the manufacturer. All purification steps were carried out at 4°C using a Pharmacia Gradifrac apparatus, unless otherwise stated, with the elution of protein continuously monitored by determining the absorbance of the eluate at 280 nm. Fractions of the eluent were collected and sampled for enzyme activities.

Frozen plant tissue was ground to a powder under liquid nitrogen using a pestle and mortar and suspended in TrisHCl (0·1 M; pH 7·5) containing ethylenediaminetetraacetic acid (EDTA; 1 mM), 2-mercaptoethanol (14 mM) and polyvinylpolypyrrolidone (75 g kg^{-1}). After filtering through two layers of muslin, the homogenate was centrifuged (5000*g*, 20 min, 4°C) and protamine sulfate added to the supernatant to a final concentration of 1·4 g litre^{-1}.[14] After re-centrifuging, a protein precipitate was obtained by adjusting the supernatant to 80% saturation with ammonium sulfate and collected by centrifugation. The pellet was dissolved in potassium phosphate buffer (10 mM; pH 7·4) containing EDTA (1 mM) 2-mercaptoethanol (14 mM) and ammonium sulfate (0·5 M) and applied to a 35-ml column of phenyl-Sepharose CL-4B (Pharmacia). After washing with loading buffer to remove unbound proteins, the column was eluted with potassium phosphate buffer (10 mM; pH 7·4; wash 1) and then with potassium phosphate buffer (10 mM; pH 7·4) containing ethylene glycol (500 g litre^{-1}) (wash 2). The active fractions from wash 2 were applied onto a 6-ml column of Fast Flow Q-Sepharose (Pharmacia) in Tris HCl (20 mM; pH 7·8) containing EDTA (1 mM) and 2-mercaptoethanol (14 mM). After removing the unbound protein and ethylene glycol, the retained proteins were eluted with a 60-ml linear gradient from 0 to 0·25 M sodium chloride at a flow rate of 1 ml min^{-1}. Fractions containing active GSTs were grouped into four definable peaks of activity which were individually dialysed against buffer A [potassium phosphate buffer (10 mM; pH 7·4) containing EDTA (1 mM) and 2-mercaptoethanol (14 mM)] and then transferred to 50-ml centrifuge tubes. *S*-hexyl-glutathione-Sepharose (0·6 g) which had been prepared as detailed previously[11] and which had been pre-equilibrated in buffer A, was added to each tube. The tubes were incubated overnight at 4°C on an end-over-end mixer and the affinity matrix then sedimented by centrifugation (4000*g*, 3 min). After decanting the supernatant, the matrix was resuspended in buffer A (10 ml) containing potassium chloride (0·2 M) and, after mixing on the end-over-end shaker for 60 min, the tubes were re-centrifuged and the supernatant removed. This washing procedure was repeated a further three times. Finally, the bound GSTs were recovered from the washed matrix by adding buffer A (0·5 ml) containing potassium chloride (0·2 M) and *S*-hexyl-glutathione (5 mM) and this eluate was dialysed against buffer A prior to assay.

To monitor the polypeptide composition of the GST preparations, active fractions were analysed by silver staining following sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12·5% acrylamide gels as described previously.[12]

2.4 Subcellular fractionation studies

Fresh, unfrozen cells were homogenised using a pestle and mortar in 60 ml of ice-cold TrisHCl (0·1 M; pH 7·5) containing EDTA (2 mM) 2-mercaptoethanol (5 mM) and sucrose (0·25 M). The resulting slurry was passed through two layers of muslin and then centrifuged (12 000*g*, 20 min, 4°C). The cell-free supernatant was then filtered through glass wool and re-centrifuged at 100 000*g* for 60 min. After decanting the cytosol, the microsomal pellet was resuspended in extraction buffer. The cell-free extract, microsomes and cytosol were then assayed for GST activity and protein content.

3 RESULTS

3.1 Metabolism of atrazine in suspension-cultured cells of *Setaria faberi*

Suspension-cultured cells of *S. faberi* were fed with 2·3 μM [*triazinyl* ^{14}C]atrazine and the uptake and metabolism of the herbicide monitored every 24 h for four days. During the feeding study, the fresh weight (FW) of the control cultures treated with 50 μl methanol increased from 2·1 g to 4·1 g, while, in the treated cultures, the final FW was 3 g, showing that the treatment with atrazine had resulted in a 50% decrease in cell growth. Similarly, the growth of non-photosynthetically active suspension cultures of celery,[16] and maize[4] have also been reported to be inhibited by similar micromolar concentrations of chloro-*s*-triazine herbicides. However, although the atrazine was phytotoxic, the radiolabel was taken up from the medium in a time-dependent manner, with an increasing proportion being associated with extractable and non-extractable residues in the cells (Table 1). The media and cell extracts were analysed by TLC and the radioactive metabolites identified by autoradiography and quantified as either unchanged [^{14}C]atrazine or metabolites derived from glutathione conjugation, which were notably *S*-atrazine-glutathione and *S*-atrazine-cysteine (data not shown) as determined in previous metabolism studies in whole plants.[13,14] Metabolites of [^{14}C]atrazine accumulated only in the cells, with the unchanged herbicide being the only radiolabelled compound determined in the medium at all time points. In the cells, glutathione conjugation was the major route of atrazine metabolism, with the small amount of radioactivity not accounted for in Table 1 being tentatively identified as hydroxyatrazine (data not shown). The time-dependent formation of the glutathione conjugation products was associated with non-extractable radioactivity in the cell debris, suggesting that this route of metabolism resulted in incorporation into insoluble biopolymers. However, the nature of this bound residue was not investigated further.

3.2 GST activities in suspension-cultured cells of *Setaria faberi*

The results of the metabolism study suggested that the *S. faberi* cultures contained GSTs active in [^{14}C]atrazine conjugation. To confirm this, GST activities toward CDNB, atrazine, the chloroacetanilides metolachlor and alachlor, and the diphenyl ether herbicide fluorodifen were determined in the cell cultures at various times after sub-culturing, and compared with the activities determined in *S. faberi* plants. The herbicides were selected as preferred substrates for differing types of plant GSTs and, in the case of atrazine and the chloroacetanilides, they are used to control *S. faberi* in the field.[13,14] The cultures began to grow rapidly two days after sub-culture and by seven days had reached stationary phase (Table 2). GST activity toward all substrates was observed at all stages of growth and was highest three days after sub-culture when cell growth was most rapid. Between day 0 and day 3 the increase in specific activity toward the chloroacetanilides was approximately 8-fold. In contrast, activities toward CDNB and atrazine only increased by 4-fold and 2-fold respectively. This differential enhancement indicated

TABLE 1
Uptake and Metabolism of [^{14}C]atrazine in Suspension-Cultured Cells of *Setaria faberi*

	Administered radioactivity[a] (%)					
Day	*Medium*	*Cell extract*	*Cell residue*	*Total*	*Atrazine*	*Atrazine-GS*[b]
1	95 (±2)	6 (±0)	0 (±0)	101	97 (±2)	1·3 (±0·2)
2	84 (±3)	8 (±1)	4 (±1)	96	88 (±3)	2·5 (±0·4)
3	80 (±5)	11 (±1)	5 (±2)	96	82 (±4)	4·5 (±0·5)
4	60 (±3)	16 (±2)	18 (±4)	94	56 (±7)	11·6 (±2·1)

[a] Values refer to the means of duplicate incubations the variation between the mean and the replicates.
[b] Atrazine-GS refers to *S*-atrazine-glutathione and its related metabolites determined in the cells and medium.

TABLE 2
Setaria faberi GST Activities in Ammonium Sulfate-Precipitated Crude Extracts from Suspension-Cultured Cells Zero to Seven Days following Sub-culture and in 10-Day-Old Seedlings

Day	Fresh weight (g)	GST activities (pkat mg^{-1} protein)[a]				
		CDNB	Atrazine	Alachlor	Metolachlor	Fluorodifen
Leaves						
10	—	100 (±40)	0·3 (±0·1)	0·4 (±0·1)	0·5 (±0·2)	0·4 (±0·0)
Cultures						
0	2·1	450 (±20)	2·8 (±0·1)	2·9 (±0·3)	2·0 (±0·1)	2·1 (±0·4)
3	4·2	1900 (±90)	5·9 (±0·4)	25·5 (±1·7)	15·6 (±2·1)	16·3 (±0·2)
5	8·8	720 (±50)	2·6 (±0·0)	3·4 (±0·2)	2·4 (±0·2)	2·0 (±0·1)
7	10·7	490 (±50)	2·7 (±0·2)	3·0 (±0·2)	1·9 (±0·0)	2·3 (±0·3)

[a] Values represent the means obtained from two flasks (± the variation in the replicates)

that the cell cultures must contain multiple GSTs of differing substrate specificity which can be differentially regulated. The GST specific activities toward all substrates was much higher in suspension-cultured cells than in 10-day old foliage (Table 2). Again, the enhancement of the GST activities toward the chloroacetanilides and fluorodifen (between 30 and 60-fold) was greater than that determined with the activities toward CDNB and atrazine (20-fold).

To determine the sub-cellular distribution of these GST activities, microsomes and cytosol were prepared from freshly harvested cells. With CDNB, the chloroacetanilides and fluorodifen as substrates, GST activities were found only in the cytosol. With atrazine as substrate over 90% of the GST activity was cytosolic, though the microsome preparation did contain measurable activity (0·12 pkat mg^{-1} protein). It was concluded that the GSTs in *S. faberi* cultures were predominantly soluble enzymes.

3.3 Resolution of GST activities toward herbicides from suspension-cultured cells of *Setaria faberi*

The observation that the GSTs in *S. faberi* cell cultures undergoing logarithmic growth contained 20- to 60-fold higher specific GST activities than those determined in plants indicated they were a good source for the purification of GST isoenzymes. Crude ammonium sulfate-precipitated protein pellets from three-day-old cultures were applied to a column of phenyl-Sepharose in 0·5 M ammonium sulphate. Under these conditions all of the GST activity toward CDNB was retained. After removing the ammonium sulfate from the eluting buffer, 8% of the applied GST activity was recovered, with a further 68% of the activity being eluted with a wash containing ethylene glycol. This latter hydrophobic GST fraction, which had been purified 18-fold (Table 3) was then applied onto a Q-Sepharose column and fractions analysed for GST activities toward CDNB and

TABLE 3
Purification of GSTs with Activity toward CDNB from Suspension-Cultured *Setaria faberi*

Purification step	Protein (mg)	Specific activity (nkat mg^{-1} protein)	Total activity (nkat)	Recovery (%)	Purification (fold)
Ammonium sulfate ppt.	686	1·9	1303	100	1
Phenyl-Sepharose	26	34·0	884	68	18
Q-Sepharose					
peak 1	1·6	53·2	85	7	28
peak 2	1·8	108·0	194	15	57
peak 3	4·7	67·4	317	24	35
S-Hexyl-glutathione agarose					
peak 1	0·01	221·5	2	0·2	117
peak 2	0·03	449·1	13	1·0	236
peak 3	0·06	260·8	16	1·2	137

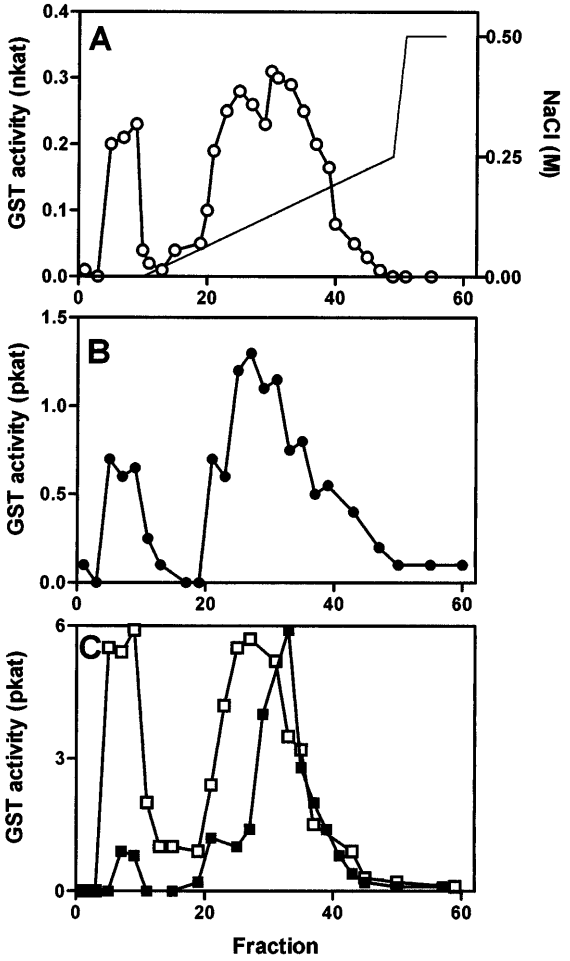

Fig. 1. Anion-exchange chromatography of partially purified GST activities toward (A) CDNB (○). (B) Atrazine (●). (C) Fluorodifen (□) and metolachlor (■). The increasing concentration of sodium chloride used to elute the activities and the numbering of the peaks is shown in (A). GST activities refer to the total activities per fraction.

the herbicides (Fig. 1). With CDNB as substrate, three peaks of GST activity could be resolved (Fig. 1A). Peak 1 (fractions 5–9) was unretained on the column, while peak 2 (fractions 20–28) and peak 3 (fractions 30–40) were recovered by increasing the salt concentration in the eluting buffer to 0·13 M sodium chloride and 0·17 M sodium chloride respectively. That peak 1 was unretained could have resulted from either overloading the column with protein, the presence of salts in the loading buffer or GST isoenzymes with no nett negative charge at pH 7·8. To examine these possibilities, a sample of the peak 1 GSTs was dialysed against 20 mM TrisHCl pH 7·8 and loaded onto a fresh Q-Sepharose column. The GST activity was again unretained. From this it was concluded that the GSTs in *S. faberi* cell cultures were composed both of acidic isoenzymes, such as those contained in peaks 2 and 3, which were retained on Q-Sepharose and the basic, unretained peak 1 GSTs. When assayed with the herbicides, the three GST peaks showed differing relative activities. Peak 1 GSTs were active toward atrazine and metolachlor but had little activity toward fluorodifen. Both peak 2 and peak 3 contained GST activities toward atrazine and metolachlor, but the majority of the activity toward fluorodifen was associated with peak 2.

3.4 Purification of GSTs by affinity chromatography

The GSTs present in *S. faberi* cultures, having been resolved into three peaks of activity, were then further purified by affinity chromatography. In view of the limiting amounts of protein present (Table 3), rather than using further column chromatography, the GSTs in each of the peaks were batch-purified by adding portions of the affinity matrix *S*-hexyl-glutathione agarose. After washing the matrix with 0·2 M potassium chloride to remove loosely bound GSTs, the affinity-bound GSTs were then recovered using *S*-hexyl-glutathione. The polypeptides present in the loosely bound and affinity-bound fractions from each of the peaks were then analysed by SDS-PAGE (Fig. 2). The affinity-

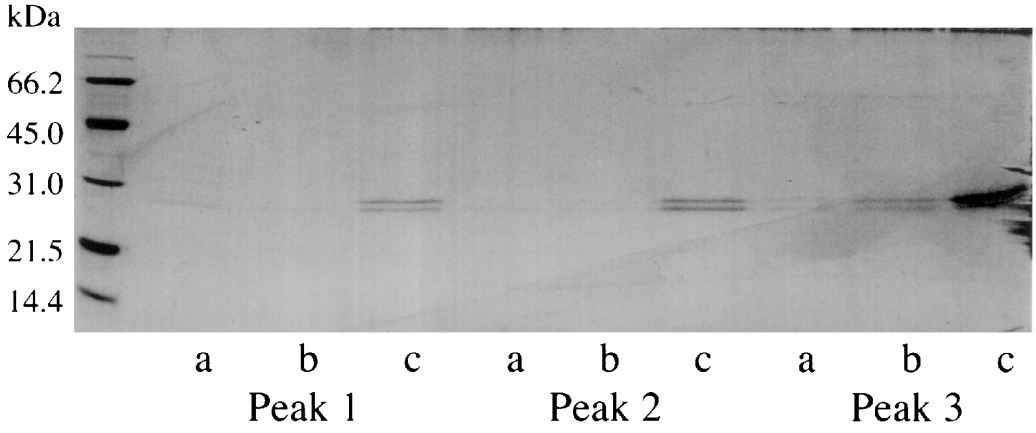

Fig. 2. Silver-stained gel of polypeptides resolved by SDS-PAGE present in the three peaks of GST activity resolved by anion-exchange chromatography following incubation with the affinity matrix *S*-hexyl-glutathione agarose. The GSTs were then recovered as either (a) unbound protein, (b) protein eluted with 0·2 M potassium chloride or (c) with 5 mM *S*-hexyl-glutathione. The left-hand lane shows reference polypeptides with their relative molecular masses indicated.

purified GSTs from each of the peaks all contained two polypeptides with estimated molecular masses of 28 kDa and 26 kDa. In peaks 1 and 2 the two polypeptides stained with a similar intensity, while in peak 3 the 28 kDa polypeptide predominated. In the loosely bound fractions from the three peaks, similar 28 kDa and 26 kDa polypeptides were just visible, suggesting that the affinity matrix may have been overloaded. Using this procedure, the affinity-purified GSTs from the peaks 1, 2 and 3 were purified 222-fold, 449-fold and 261-fold respectively, though in each case the final recoveries were low (Table 3). The low recoveries resulted in a large proportion of the GST activity toward CDNB being unretained on the *S*-hexyl-glutathione agarose. This appeared to be due to the matrix being overloaded, as these unbound GSTs were retained when re-applied onto fresh *S*-hexyl-glutathione agarose.

4 DISCUSSION

The GSTs of suspension-cultured cells of *S. faberi* exhibited a similar range of GST activities toward CDNB and herbicides to that determined in the foliage of the plant, but in actively dividing cells the specific activities (pkat mg^{-1} protein) were between 20- and 60-fold higher than in the plants. As compared with plants, GST activities toward CDNB have also been reported to be enhanced in de-differentiated cultures of maize, soybean and pumpkin.[4,7,9]. This enhancement largely results from the presence of the auxin 2,4-dichlorophenoxyacetic acid in the culture medium, which causes the enhancement of GSTs normally only determined at low levels in plant cells, together with the induction of novel isoenzymes.[17] Thus, it has been reported that, when 2,4-D is removed from the culture medium, the specific activity towards CDNB was reduced in pumpkin callus cultures.[9] In the *S. faberi* cultures all the GST activities underwent a large, but transient, increase during the most active period of cell division. This suggests that GSTs with undefined functions which accumulate during cell division[17] are active in herbicide metabolism.

In contrast to earlier studies in maize suspension cultures,[4,5] the *S. faberi* cells expressed GSTs capable of conjugating atrazine. Thus, unlike Black Mexican Sweetcorn cultures, the *S. faberi* cells readily conjugated atrazine with glutathione. Interestingly, with the exception of *Zm* GST I-I isoenzyme, atrazine conjugation is not associated with any of the maize GSTs described to date, while in extracts from the *S. faberi* cultures this activity was found in the three peaks of GST activity resolved by anion-exchange chromatography. Previous studies have also suggested that there are significant differences in the GSTs involved in atrazine metabolism in maize and in *S. faberi*, as this conjugating activity is more susceptible to competitive inhibition by the glutathione conjugate of the atrazine synergist tridiphane in the weed than in maize.[18]

The results of the anion-exchange chromatography showed that the cell cultures contained one basic and two acidic groups of GSTs, with the complex elution profiles of the GST activities toward the individual substrates indicating a greater level of isoenzyme complexity. In comparison, four acidic major peaks of GST activity could be determined in suspension-cultured Black Mexican Sweetcorn.[6] The significance of the basic group of GSTs in the *S. faberi* cultures, as compared with the two acidic groups of isoenzymes which were also determined in *S. faberi* plants,[19] was unclear. The basic GSTs may represent isoenzymes which are only expressed following exposure to auxins, or they may result from post-translational modifications to the acidic isoenzymes. In any event, even though the three GST isoenzyme groups in the cell cultures had differing activities toward the herbicides, they all contained similar 28 kDa and 26 kDa polypeptides. It will now be of interest to characterise further the herbicide substrate specificity of these GST isoenzymes of *S. faberi* and compare them with that determined for the GSTs in maize.[11,12]

ACKNOWLEDGEMENT

This study was supported by a Co-operative Award in Science and Engineering studentship awarded to Pamela Hatton by the Environmental and Physical Sciences Research Council and Rhône-Poulenc Agriculture Ltd (RPAL) and a LINK grant awarded to Ian Cummins and Robert Edwards by the Biotechnology and Biological Sciences Research Council and RPAL.

REFERENCES

1. Mumma, R. O. & Davidonis, G. H., Plant tissue culture and pesticide metabolism. In *Progress in Pesticide Biochemistry and Toxicology. Vol. 3*. ed. D. H. Hutson & T. R. Roberts. Wiley, Chichester, 1983, pp. 255–74.
2. Sandermann, H, Jr, Scheel, D. & Trenck, T. V. D., Use of plant cell cultures to study the metabolism of environmental chemicals. *Ecotoxicol. Environ. Safety*, **8** (1984) 167–82.
3. Cole, D. J., Cummins, I., Hatton, P. J., Dixon, D. & Edwards, R., Glutathione transferases in crops and major weeds. In *Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants*, ed. K. K. Hatzios. Kluwer Academic Press, Dordrecht, 1997, pp. 139–54.
4. Edwards, R. & Owen, W. J., Comparison of glutathione *S*-transferases of *Zea mays* responsible for herbicide detoxification in plants and suspension-cultured cells. *Planta*, **169** (1986) 208–15.
5. Edwards, R. & Owen W. J., Regulation of glutathione *S*-transferases of *Zea mays* in plants and cell cultures. *Planta*, **175** (1988) 99–106.

6. Miller, K. D., Iryzk, G. P. & Fuerst, E. P., Benoxacor treatment increases glutathione *S*-transferase activity in suspension cultures of *Zea mays*. *Pestic. Biochem. Physiol.*, **48** (1994) 123–34.
7. Andrews, C. J., Skipsey, M., Townson, J. K., Morris, C., Jepson, I. & Edwards, R., Glutathione transferase activities toward herbicides used selectivity in soybean. *Pestic. Sci.*, **51** (1997) 213–22.
8. Knörzer, O. C., Durner, J. & Böger, P., Alterations in the antioxidative system of suspension-cultured soybean cells (*Glycine max*) induced by oxidative stress. *Physiol. Plant.*, **97** (1996) 388–96.
9. Fujita, M., Adachi, Y. & Hanada, Y., Preliminary characterisation of glutathione *S*-transferases that accumulate in callus cells of pumpkin (*Cucurbita maxima* Duch.). *Plant Cell Physiol.*, **35** (1994) 275–82.
10. Droog, F. R. N., Hooykaas, P. J. J. & van der Zaal, B. J., 2,4-Dichlorophenoxyacetic acid and related chlorinated compounds inhibit two auxin-regulated type-III tobacco glutathione *S*-transferases. *Plant Physiol.*, **107** (1995) 1139–46.
11. Dixon, D. P., Cole, D. J. & Edwards, R., Purification, regulation and cloning of a glutathione transferase (GST) from maize resembling the auxin-inducible type-III GSTs. *Plant Mol. Biol.*, **36** (1998) 75–87.
12. Dixon, D., Cole, D. J. & Edwards, R., Characterisation of multiple glutathione transferases with activities toward herbicide substrates in maize (*Zea mays*). *Pestic. Sci.*, **50** (1997) 72–82.
13. Hatton, P. J., Dixon, D., Cole, D. J. & Edwards, R., Glutathione transferase activities and herbicide selectivity in maize and associated weed species. *Pestic. Sci.*, **46** (1996) 267–75.
14. Hatton, P. J. Cole, D. J. & Edwards, R., Influence of plant age on glutathione levels and glutathione transferases involved in herbicide detoxification in corn (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herrm.). *Pestic. Biochem. Physiol.*, **54** (1996) 199–209.
15. Murashige, T. & Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, **15** (1962) 473–9.
16. Metcalf, E. C. & Collin, H. A., The effect of simazine on the growth and respiration of a cell suspension culture of celery. *New Phytol.*, **81** (1978) 243–8.
17. Marrs, K. A., The functions and regulation of glutathione *S*-transferases in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **47** (1996) 127–58.
18. Lamoureux, G. L. & Rusness, D. G., Tridiphane [2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane] an atrazine synergist: enzymatic conversion to a potent glutathione *S*-transferase inhibitor. *Pestic. Biochem. Physiol.*, **26** (1986) 323–42.
19. Hatton, P. J., Edwards, R., Cole, D. J. & Price, L. J., Glutathione transferases responsible for herbicide metabolism in *Setaria faberi*. *Proc. Brighton Crop Protect. Conf.—Weeds*, **1** (1995) 281–6.